References:


Der f 1 ELISA kit (6A8/4C1)

Product Code: EL-DF1
Lot Number: xxxxx

Sample Curve:

<table>
<thead>
<tr>
<th>Concentration (ng/mL)</th>
<th>Measured O.D. (405 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>0.3</td>
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<tr>
<td>1</td>
<td>2.5</td>
</tr>
<tr>
<td>10</td>
<td>2.0</td>
</tr>
<tr>
<td>100</td>
<td>1.5</td>
</tr>
<tr>
<td>1000</td>
<td>1.0</td>
</tr>
<tr>
<td>10000</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Content:

Vial 1 (red top) 200 µl
Monoclonal antibody 6A8
Concentration: 2mg/ml in PBS

Vial 2 (white top) 2 x 400 µl
Der f 1 Standard
Concentration: 2500ng/ml

Vial 3 (brown) 200 µl
Biotinylated monoclonal antibody 4C1
Dilute: 1:1000 for use

Storage: All reagents should be stored at 4°C
Certificate of Analysis

Monoclonal Antibody: 6A8 (clone 6A8 B10 D12)
Immunogen: Der f 1
Isotype: Mouse IgG1
Specificity: Binds to common epitope present on *Dermatophagoides farinae* allergen, Der f 1.
Purification: Produced in tissue culture by hollow fiber fermentation and purified by chromatography using Protein A. Single heavy and light chain bands on SDS-PAGE.
Concentration: 2.0 mg/ml in phosphate buffered saline, pH 7.2.
Lot Number: xxxxx

Monoclonal Antibody: 4C1 (clone 4C1 B8 3F8)
Immunogen: Der f 1
Isotype: Mouse IgG1
Specificity: Binds to a common epitope on mite *Dermatophagoides* Group 1 allergens (Der f 1, Der p 1, Der m 1, Eur m 1).
Purification: By HPLC using recombinant protein A. Single heavy and light chain bands on SDS-PAGE
Biotinylation: Biotinylated using EZ-Link Sulfo-NHS-LC Biotinylating Agent and titrated for use in ELISA for mite Group 1 allergen at 1/1000 dilution. Prepared in 1% BSA-50% glycerol/PBS.
Lot Number: xxxxx

Allergen Standard: Universal Allergen Standard
Composition: A formulation of eight purified natural allergens prepared in 1% BSA/50% glycerol/PBS, pH 7.4
Lot Number: xxxxx

<table>
<thead>
<tr>
<th>Universal Allergen Standard</th>
<th>Protein Measurement</th>
<th>Concentration (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Der p 1</td>
<td>Amino-acid analysis</td>
<td>2500</td>
</tr>
<tr>
<td>Der f 1</td>
<td>Amino-acid analysis</td>
<td>2500</td>
</tr>
<tr>
<td>Der p 2</td>
<td>Amino-acid analysis</td>
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<td>Fel d 1</td>
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</tr>
<tr>
<td>Can f 1</td>
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</tr>
<tr>
<td>Rat n 1</td>
<td>Amino-acid analysis</td>
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<tr>
<td>Mus m 1</td>
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<td>250</td>
</tr>
<tr>
<td>Bla g 2</td>
<td>Amino-acid analysis</td>
<td>2500</td>
</tr>
</tbody>
</table>

ELISA protocol for Der f 1.

1. Anti-Der f 1 mAb 6A8 is supplied HPLC purified as a stock solution at 2mg/ml in PBS (Product Code: MA-6A8). Dilute the mAb 6A8 1/1000 (i.e., 10µl/10ml) in 50mM carbonate-bicarbonate buffer, pH 9.6. Coat polystyrene microtiter wells (NUNC Maxisorp Cert. NUNC catalog # 439454, Fisher Catalog #12566135) with 100µl of the diluted mAb 6A8 per well. Incubate overnight at 4°C.

2. Wash wells 3x with PBS-0.05% Tween 20, pH 7.4 (PBS-T). Incubate for 30 min. at room temperature with 100µl 1% BSA PBS-T. Wash 3x with PBS-T.

3. Add 100µl of diluted allergen standard, house dust or air filter samples. Incubate for 1 hour at room temperature.

3a. Make a Der f 1 control curve using doubling dilutions of the allergen standard (Product Code: ST-DF1): The control curve dilutions are from 250 - 0.5ng/ml Der f 1. Pipette 20µl Der f 1 standard into 180µl 1% BSA-PBS-T into wells A1 and B1 of the ELISA plate. Mix well and transfer 100µl across the plate into 100µl 1% BSA PBS-T diluent to make 10 serial doubling dilutions. Wells A11, B11, A12 and B12 should contain only 1% BSA PBS-T as blanks.

3b. House dust samples are routinely diluted two-fold from 1/10-1/80.

4. Wash wells 3x with PBS-T and add 100µl diluted biotinylated anti-Group 1 mAb 4C1 (Product Code: BI-4C1). The antibody solution contains 50% glycerol and should be diluted 1/1000 (i.e., 10µl/10ml) in 1% BSA-PBST. Incubate for 1 hour at room temperature.

5. Wash wells 3x with PBS-T and add 100µl diluted Streptavidin - Peroxidase (Sigma S5512, 0.25mg reconstituted in 1 ml distilled water). The reconstituted Streptavidin should be diluted 1/1000 (i.e., 10µl/10ml) in 1% BSA PBS-T. Incubate for 30 minutes at room temperature.

6. Wash wells 3x with PBS-T and develop the assays by adding 100µl 1mM ABTS in 70mM citrate phosphate buffer, pH 4.2 containing a 1/1000 dilution of 30% H2O2 (i.e., 10µl/10ml ABTS). Read the plate when the optical density at 405nm reaches 2.0-2.4.

Notes:
The Der f 1 standard 2500ng/ml Der f 1 was sub-standardized against the WHO/IUIS *D. pteronyssinus* reference using a cross-reacting RIA (as yet, there is no International Reference Preparation of *D. farinae*).

Buffer recipes, storage conditions and a list of frequently asked questions can be found under “Protocols” on our web site: www.inbio.com.